

Pathogenic Variation of South American *Phakopsora pachyrhizi* Populations Isolated from Soybeans from 2010 to 2015

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Abstract

Soybean rust caused by *Phakopsora pachyrhizi* is one of the most serious economic threats to soybean production in South America. A previous study using South American *P. pachyrhizi* populations collected between 2007/2008 and 2009/2010 revealed the pathogenic diversity in Argentinean, Brazilian, and Paraguayan rust populations. Because such pathogenic diversity has been a major constraint to the breeding program for soybean rust resistance, pathogen populations were continuously monitored throughout the 2010/2011 to 2014/2015 seasons using the same method of evaluating pathogenicity as used in the previous study. None of the 83 *P. pachyrhizi* samples collected from the three countries from 2010/2011 to 2014/2015 yielded identical pathogenicity patterns in the 16 differentials, thus demonstrating the pathogenic diversity of more recent South American rust populations. Cluster analysis using a total of 145 rust populations from 2007 to 2015 demonstrated that the Argentinean, Brazilian, and Paraguayan populations were not assigned to three distinct country-based groups. The analysis indicated that a majority of South American populations differed in pathogenicity compared with Japanese rust races. The rates of resistance to the rust populations varied among the 13 differentials carrying *Rpp* genes; the most effective resistance gene was *Rpp1-b* followed by *Rpp5*, and the least effective was *Rpp1*.

Discipline: Plant Disease

Additional key words: Asian soybean rust, *Glycine max*, infection type, urediniospore, virulence

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Introduction

Soybean [*Glycine max* (L.) Merrill] production in South America has been growing markedly since the late 1990s, and South American countries are the largest soybean producers in the world (FAOSTAT 2013). Soybeans are economically important as a valuable source of oil and protein worldwide, and for traditional food products of the Orient. Therefore, various countries, particularly those in which the soybean industry and consumption are highly dependent on imports from other countries, are particularly interested in soybean production. With an increase in soybean cultivation areas in South America, biotic stressors such as plant pathogens and pests that negatively affect crop production are a major concern. One of the most damaging diseases of soybeans is soybean rust caused by *Phakopsora pachyrhizi* H. Sydow & P. Sydow (Hartman et al. 2011, Miles et al. 2003, Pennisi 2010). Soybean rust can result in a 10%-80% reduction in yield or losses of over 80%, particularly when the disease develops under preferable environmental conditions for *P. pachyrhizi* (Hartman et al. 2005, Levy 2005, Li et al. 2010, Pennisi 2010, Rosa et al. 2015, Yorinori et al. 2005). Since the first outbreak of soybean rust caused by *P. pachyrhizi* in Paraguay and Brazil in 2001 and in Argentina in 2003 (Ivancovich 2005, Rossi 2003, Yorinori et al. 2005), it has rapidly spread to most of the soybean fields throughout South America, and has continuously impacted soybean production in the region, resulting in tremendous economic losses.

Although the use of fungicides is a major measure to control the disease, production costs increase considerably with such use due to the cost of the chemicals and application management (Godoy et al. 2016). Furthermore, reduced sensitivity of rust isolates to the fungicides has been observed in Brazil (Godoy et al. 2015), most likely due to the extensive and repeated use of fungicides. Using rust-resistant soybean varieties is more economical and environment friendly than chemical control methods. Past researches on host resistance to soybean rust have identified six major resistance loci (*Rpp*: resistance to *P. pachyrhizi*), named *Rpp1* to *Rpp6* and an allele (Chakraborty et al. 2009, Garcia et al. 2008, Hartwig 1986, Hartwig & Bromfield 1983, Li et al. 2012, McLean & Byth 1980, Ray et al. 2009). These *Rpp* genes confer specific resistance to a limited set of *P. pachyrhizi* isolates, some of which have been used to develop *Rpp* gene-carrying cultivars (Garcia et al. 2011, Hartman et al. 2011) and *Rpp*-pyramided lines (Lemos et al. 2011, Maphosa et al. 2012, Yamanaka et al. 2015b) using conventional strategies.

By using such differences in the host-plant responses,

a method of evaluating soybean resistance to *P. pachyrhizi* and assessing the pathogenicity of *P. pachyrhizi* has been established (Akamatsu et al. 2013, Yamanaka et al. 2010). In addition, *P. pachyrhizi* samples from Argentina, Brazil, and Paraguay from the 2007/2008-2009/2010 cropping seasons were tested for pathogenicity on 16 soybean differentials (Akamatsu et al. 2013). The report showed a substantial pathogenic variation in the rust populations from South America and useful resistance genes. Knowledge about the pathogenic variation of the fungus is necessary for identifying genetic sources useful for reducing disease and developing rust-resistant soybean cultivars for specific growing regions.

The objectives of this study were (1) to investigate the pathogenicity of *P. pachyrhizi* populations infecting soybeans in Argentina, Brazil, and Paraguay from 2010 to 2015 and (2) to compare rust pathogenicity data from total eight cropping seasons from 2007 to 2015. This paper provides valuable knowledge regarding characteristics of soybean rust pathogenicity in South America that can be used to identify resistant soybean genotypes and target effective cultivars against certain pathogen populations.

Materials and methods

1. Soybean rust sampling in three South American countries

Soybean rust samples were collected in Argentina, Brazil, and Paraguay during five cropping seasons from 2010/2011 to 2014/2015 (Table 1). The table includes the soybean rust samples collected in a previous study conducted during the three cropping seasons of 2007/2008, 2008/2009, and 2009/2010 (Akamatsu et al. 2013). *P. pachyrhizi* population samples without single-spore (or single-lesion) isolation were used in the present study to investigate the pathogenic differences among the dominant races, rather than specific races in the samples, and to compare the pathogenicity data with the previous study (Akamatsu et al. 2013). Seven Japanese rust isolates collected in 2007, 2008, and 2009 were also used to compare pathogenicity with the South American rust samples. Each of these Japanese isolates belongs to individual races, representing seven out of eight races that were found in Ibaraki Prefecture, Japan (Table 1; Yamaoka et al. 2014). The harvesting of urediniospores from diseased soybean leaves and their storage until use were performed according to Akamatsu et al. (2013) and Yamanaka et al. (2013).

2. South American soybean rust collection from 2010/2011 to 2014/2015

In Argentina, 28 soybean rust populations were

collected from 17 locations in 10 provinces during the five cropping seasons from 2010/2011 to 2014/2015 (Table 1). The *P. pachyrhizi* sample codes consisted of the sampling location codes and cropping season numbers. The 2007/2008 season was defined as the first season of the sampling period in the present study, and the numbers

for the 2010/2011, 2011/2012, 2012/2013, 2013/2014, and 2014/2015 cropping seasons correspond to 4, 5, 6, 7, and 8, respectively. The location codes APM, ANE, and ANW denote the Argentina-Pampa, Argentina-Northeast, and Argentina-Northwest regions, respectively. According to this coding system, APM1-5 is a sample collected

Table 1. Soybean rust samples used in this study

Country	N o .	Location	Sample code							
			Soybean croppingseason							
			2007/2008	2008/2009	2009/2010	2010/2011	2011/2012	2012/2013	2013/2014	2014/2015
Argentina ^a	1	Pergamino, Buenos Aires	APM1-1 ^b	—	APM1-3	—	APM1-5 ^b	APM1-6 ^b	APM1-7	APM1-8 ^b
	2	Acebal, Santa Fé	—	APM2-2 ^b	APM2-3 ^b	—	—	—	—	—
	3	Coronel Bogado, Santa Fé	—	—	APM3-3 ^b	—	—	—	—	—
	4	Rosario, Santa Fé	—	—	APM4-3 ^b	—	APM4-5 ^b	—	—	—
	5	Villa Mantero, Entre Ríos	—	—	APM5-3 ^b	—	—	—	—	—
	6	Cerro Azul, Misiones	—	ANE6-2 ^b	ANE6-3 ^b	—	ANE6-5 ^b	ANE6-6	ANE6-7	—
	7	El Colorado, Formosa	ANE7-1 ^b	—	ANE7-3 ^b	ANE7-4 ^b	—	ANE7-6 ^b	ANE7-7	—
	8	Saenz Peña, Chaco	ANE8-1 ^b	—	—	—	—	ANE8-6 ^b	—	—
	9	Pozo Hondo, Santiago del Estero	—	—	ANW9-3 ^b	—	—	—	—	—
	10	Alderete, Tucumán	—	—	ANW10-3 ^b	—	—	—	—	—
	11	Metán, Salta	—	—	ANW11-3 ^b	—	—	—	—	—
	12	Ledesma, Jujuy	—	ANW12-2 ^b	—	—	ANW12-5 ^b	—	ANW12-7	—
	13	Sunchales, Santa Fé	—	—	—	APM13-4 ^b	—	—	—	—
	14	Humboldt, Santa Fé	—	—	—	APM14-4 ^b	—	—	—	—
	15	Esperanza, Santa Fé	—	—	—	—	—	—	APM15-7	—
	16	Gálvez, Santa Fé	—	—	—	—	—	—	—	APM16-8 ^b
	17	Reconquista, Santa Fé	—	—	—	APM17-4 ^b	—	—	APM17-7	—
	18	Paraná, Entre Ríos	—	—	—	—	—	—	APM18-7	APM18-8 ^b
	19	La Paz, Entre Ríos	—	—	—	—	—	APM19-6 ^b	—	APM19-8 ^b
	20	Marcos Juárez, Córdoba	—	—	—	—	—	—	APM20-7	—

	21	Esquina, Corrientes	—	—	—	—	—	ANE21-6	—	—
	22	El Sombrero, Corrientes	—	—	—	—	—	—	ANE22-7	—
	23	Pichanal, Salta	—	—	—	—	—	—	ANW23-7	—
Brazil	1	Passo Fundo, Rio Grande do Sul (RS)	BSO1-1	BSO1-2	BSO1-3	—	—	BSO1-6 ^b	BSO1-7 ^b	BSO1-8
	2	Londrina, Paraná (PR) ^c	BSO2-1 ^b	BSO2-2	BSO2-3	BSO2-4(1) BSO2-4(2)	BSO2-5	BSO2-6	BSO2-7 ^b	BSO2-8
	3	Londrina, Paraná (PR) ^d	BSO3-1 BRP-1 ^{b, e} BRP-2 ^{b, e}	—	—	BSO3-4(1) BSO3-4(2)	BSO3-5(1) BSO3-5(2) BSO3-5(3) BSO3-5(4)	BSO3-6	BSO3-7	BSO3-8 ^b
	4	Uberaba, Minas Gerais (MG)	—	BSO4-2	—	—	—	—	—	—
	5	Dourados, Mato Grosso do Sul (MS)	BCW5-1(1) ^f BCW5-1(2) ^f	—	BCW5-3 ^b	BCW5-4	—	—	—	—
	6	Chapadão do Sul, MS	BCW6-1	—	—	BCW6-4 ^b	—	—	BCW6-7 ^b	BCW6-8
	7	Campo Verde, Mato Grosso (MT)	BCW7-1	—	—	—	—	BCW7-6 ^b	—	—
	8	Rondonópolis, MT	—	—	BCW8-3 ^b	—	—	—	—	BCW8-8
	9	Goiânia, Goiás (GO)	BCW9-1	—	—	BCW9-4 ^b	—	—	—	—
	10	Senador Canedo, GO	BCW10-1	—	BCW10-3 ^g	—	BCW10-5	—	—	—
	11	Planaltina, Distrito Federal (DF)	BCW11-1	BCW11-2	BCW11-3	—	BCW11-5	—	—	—
	12	Vilhena, Rondônia (RO)	BNO12-1	BNO12-2	BNO12-3	—	BNO12-5	—	—	—
	13	São Raimundo das Mangabeiras, Maranhão (MA)	—	BNO13-2 ^g	—	—	—	—	—	—
	14	São Desidério, Bahia (BA)	—	BNO14-2 ^g	—	—	—	—	—	—
	15	Rio Verde, GO	—	—	—	—	BCW15-5	—	BCW15-7	—
	16	Primavera do Leste, MT	—	—	—	—	—	—	BCW16-7	—
Paraguay	1	Corpus Christi, Canindeyú	PNC1-1	—	—	—	—	—	—	—
	2	Pindoty Porã, Canindeyú	PNC2-1	—	—	—	—	—	—	—
	3	Katueté, Canindeyú	—	—	PNC3-3	PNC3-4	PNC3-5 ^b	—	—	PNC3-8
	4	Troncal, Alto Paraná	—	—	PMA4-3	—	—	—	—	—
	5	Minga Porã, Alto Paraná	—	—	PMA5-3	—	—	—	—	—

6	San Alberto, Alto Paraná	PMA6-1	PMA6-2	—	—	PMA6-5 ^b	PMA6-6	PMA6-7	PMA6-8
7	Itakyry, Alto Paraná	—	—	PMA7-3	PMA7-4 ^b	—	—	—	—
8	Santa Rita, Alto Paraná	—	PMA8-2	—	—	—	—	—	—
9	Naranjal, Alto Paraná	PMA9-1	—	—	—	—	—	—	—
10	Naranjito, Itapúa	PSI10-1	—	—	—	—	—	—	—
11	Maria Auxiliadora, Itapúa	—	—	PSI11-3	—	—	—	—	—
12	Capitán Meza, Itapúa	—	PSI12-2	—	PSI12-4 ^b	—	—	—	—
13	Pirapó, Itapúa	PSI13-1	PSI13-2	—	PSI13-4 ^b	PSI13-5 ^b	PSI13-6	PSI13-7	PSI13-8
14	Bella Vista, Itapúa	—	—	PSI14-3	—	—	—	—	—
15	Capitán Miranda, Itapúa	PSI15-1	PSI15-2	PSI15-3	PSI15-4	PSI15-5	PSI15-6	PSI15-7	PSI15-8
16	Choré, San Pedro	—	—	—	—	—	PNS16-6	—	PNS16-8
17	Yhový, Canindeyú	—	—	—	—	—	—	—	PNC17-8
18	Yguazú, Alto Paraná	—	—	—	—	—	—	—	PMA18-8
19	Dr. Juan Eulogio Estigarribia, Caaguazú	—	—	—	—	—	—	—	PMG19-8
Japan	—	NICS, Kannon-dai, Tsukuba, Ibaraki	T1-4 ^h N1-1 ^h E1-4 ^h						
	—	Kannon-dai, Tsukuba, Ibaraki	K1-2 ^{h,i}						
	—	Kurihara, Tsukuba, Ibaraki		N2-3 ^{b,j} T3-1 ^j					
	—	Azuma, Tsukuba, Ibaraki		K3-1 ^{i,k}					

^a The location numbers correspond to those in Tables 2, 3, and 4.

^b The rust samples were not evaluated using a complete set of 16 differentials due to preparation failure involving certain differential plants.

^c The rust samples except for BSO2-4(2) were isolated from a field of EMBRAPA Soja, Londrina, PR, Brazil. BSO2-4(2) was isolated from a field near EMBRAPA Soja.

^d The rust samples were isolated from the greenhouses of EMBRAPA Soja, Londrina, PR, Brazil.

^e Yamanaka et al. (2010) and Akamatsu et al. (2013).

^f Two samples were isolated on two different dates in the 2007/2008 season (Akamatsu et al. 2013).

^g Three samples were collected in the 2007/2008-2009/2010 seasons, but the evaluation data was not included in Akamatsu et al. (2013).

^h The rust samples were isolated in 2007 (Yamaoka et al. 2014).

ⁱ Soybean rust isolates K1-2 and K3-1 were collected from kudzu (Yamaoka et al. 2014).

^j The rust sample was isolated in 2008 (Yamaoka et al. 2014).

^k The rust sample was isolated in 2009.

from Pergamino, Buenos Aires, Argentina-Pampa region during the 2011/2012 season (the fifth season).

In Brazil, 31 rust populations were collected from six states and the Federal District (Distrito Federal, DF; Table 1). The location codes BSO, BSE, BCW, BNO, and BNE denote the Brazil-South, Brazil-Southeast, Brazil-Central-West, Brazil-North, and Brazil-Northeast regions, respectively. BSE and BNE coded for the populations from the 2007/2008–2009/2010 seasons were treated as BSO and BNO in this study.

In Paraguay, 24 rust populations were sampled from five departments (Table 1). The location codes PNC, PNS, PMA, PMG, and PSI denote the Paraguay-Eastern region North-Canindeyú, Paraguay-Eastern region North-San Pedro, Paraguay-Eastern region Middle-Alto Paraná, Paraguay-Eastern region Middle-Caaguazú, and Paraguay-Eastern region South-Itapúa prefectures, respectively.

3. Differential plants, inoculation, and data collection

The same soybean differential set was used as in our previous study (Akamatsu et al. 2013, Yamanaka et al. 2013). The procedures for rust urediniospore multiplication, soybean inoculation, and pathogenicity evaluation were adopted from those that we used previously (Akamatsu et al. 2013, Yamanaka et al. 2013). The reaction was classified into three categories: 1) R, immune or resistant; 2) IM, intermediate; and 3) S, susceptible.

4. Data analysis

The R, IM, and S infection types were coded as 0, 1, and 2, respectively. The distance matrices were prepared by calculating Euclidean and the squared Euclidean distances between samples using R software version 3.2.0 (R Core Team 2015). Dendrograms based on the unweighted paired group method with an arithmetic mean (UPGMA) were also constructed with R. An R package (pvclust) was run to assess the uncertainty in hierarchical cluster analysis, which calculates approximately unbiased probability values (AU p-values) for each cluster using multiscale bootstrap resampling techniques (Suzuki & Shimodaira 2006).

Results

1. Pathogenicity of the *Phakopsora pachyrhizi* population from Argentina during the 2010/2011 to 2014/2015 seasons

In the 28 rust samples, no identical pathogenicity patterns within the 16 differentials were observed (Table 2). Two populations, ANE7-4 and ANE7-6, exhibited

identical pathogenicity patterns in 12 differentials. In addition, 14 reaction matches out of 15 comparable differentials were identified in APM18-7 and APM18-8. These samples were derived from the same location in the Northeast and Pampa regions, respectively, but during different seasons. The pathogenicity profiles differed among the multiple rust samples collected at each of the seven locations during the five different cropping seasons (APM1, ANE6, ANE7, ANW12, APM17, APM18, and APM19). Thus, no strong association was observed in the pathogenicity profiles of Argentinean rust populations during the 2010/2011–2014/2015 seasons with the geographical origins of the isolation. ANE6-6 displayed the highest virulence in the 16 differentials in Argentina, none of which induced resistant reactions against the samples.

2. Pathogenicity of the *Phakopsora pachyrhizi* population from Brazil during the 2010/2011 to 2014/2015 seasons

No identical pathogenicity patterns among the 16 differentials were found in the 31 *P. pachyrhizi* samples collected from Brazil (Table 3). The highest level of similarity in the pathogenicity profiles of the 16 differentials was found between BSO3-5(2) and BSO3-5(4) with 15 reaction matches, followed by BCW8-8 and BCW15-7 with 14 reaction matches. Pathogenic variations were detected among rust samples collected from the same location (BSO1, BSO2, BSO3, BCW6, and BCW15), but were from different seasons although certain sample pairs (e.g., BSO2-6 and BSO2-8, BCW6-7 and BCW6-8) induced identical reactions to 13 out of 15 or 16 differentials. Pathogenicity also varied among rust samples with the same regional (South and Central-West regions) and seasonal origins, indicating no apparent region-specific pathogenicity in Brazil. BSO3-7 was the most virulent of the Brazilian rust populations. It did not cause any resistant reactions in the 16 differentials. BSO2-6 and BSO3-6 also displayed high virulence in 13 out of 16 differentials.

3. Pathogenicity of the *Phakopsora pachyrhizi* population from Paraguay during the 2010/2011 to 2014/2015 seasons

All rust samples from Paraguay differed in the level of pathogenicity towards the 16 differentials (Table 4). The highest similarity of the pathogenicity profiles out of 24 Paraguayan samples was detected between PSI13-8 and PMG19-8, which only caused different reactions with PI 587886 (differential 11). PSI15-6 was the most virulent of the differentials in the rust populations from Paraguay, inducing resistant reactions in only two

Rpp-carrying differentials (differentials 7 and 8). In contrast, a few rust samples such as PSI13-7 exhibited lower pathogenicity towards the differentials. However, PMA6-7 never produced a susceptible reaction in any of the 13 *Rpp*-carrying differentials. Variation in the

pathogenic characteristics of the sampling locations was not observed in Paraguay, as the pathogenicity profiles differed among rust samples collected from the same location (PNC3, PMA6, PSI13, PSI15, and PNS16), but during different seasons.

Table 2. Infection types of 28 *Phakopsora pachyrhizi* samples from Argentina on 16 differentials ^a

Location no. ^b	1				4	6				7				8	12	13	14	15	16	17		18		19	20	21	22	23
Season	2011/ 2012	2012/ 2013	2013/ 2014	2014/ 2015	2011/ 2012	2011/ 2012	2012/ 2013	2013/ 2014	2010/ 2011	2012/ 2013	2013/ 2014	2012/ 2013	2011/ 2012	2013/ 2014	2010/ 2011	2010/ 2011	2013/ 2014	2014/ 2015	2010/ 2011	2013/ 2014	2013/ 2014	2014/ 2015	2012/ 2013	2014/ 2015	2013/ 2014	2012/ 2013	2013/ 2014	
Sample code	APM1-5	APM1-6	APM1-7	APM1-8	APM4-5	ANE6-5	ANE6-6	ANE6-7	ANE7-4	ANE7-6	ANE7-7	ANE8-6	ANW12-5	ANW12-7	APM13-4	APM14-4	APM15-7	APM16-8	APM17-4	APM17-7	APM18-7	APM18-8	APM19-6	APM19-8	APM20-7	ANE21-6	ANE22-7	ANW23-7
1 PI 200492	S	S	S	S	S	S	S	S	S	nd	R	nd	S	S	S	S	S	nd	S	S	S	S	S	S	S	S	S	S
2 PI 368039	S	S	S	S	S	S	S	S	S	S	IM	S	S	S	S	S	S	nd	S	S	R	nd	S	nd	S	IM	S	IM
3 PI 230970	nd	S	IM	S	S	nd	IM	S	S	S	IM	S	nd	IM	IM	S	S	S	R	IM	R	R	S	R	IM	R	S	S
4 PI 417125	S	S	R	S	S	R	IM	S	nd	R	S	S	R	R	R	R	S	R	nd	IM	R	R	nd	S	S	S	R	R
5 PI 462312	S	S	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	S	R	R	R	R	R
6 PI 459025	nd	R	R	S	nd	nd	S	R	S	S	R	R	nd	R	nd	nd	R	S	S	R	R	R	R	R	R	R	R	R
7 Shiranui	S	IM	R	nd	R	R	IM	IM	S	S	IM	S	R	R	S	nd	R	R	IM	S	R	R	R	R	R	R	S	IM
8 PI 416764	S	nd	S	S	nd	IM	S	S	nd	nd	S	S	IM	S	nd	nd	S	S	nd	S	S	S	S	S	S	IM	S	S
9 PI 587855	S	R	R	R	R	S	IM	R	R	nd	R	R	R	IM	R	R	R	R	R	R	R	R	R	nd	R	R	R	R
10 PI 587880A	R	R	R	R	R	S	S	IM	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
11 PI 587886	nd	S	S	S	S	S	S	S	S	S	S	IM	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
12 PI 587905	S	S	R	R	R	nd	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	IM
13 PI 594767A	R	S	R	R	R	S	S	R	R	R	IM	R	R	R	R	R	R	R	nd	R	R	R	R	R	R	S	R	R
14 BRS 154	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	nd	S	S	IM	S	S	IM	S	IM	S
15 TK5	S	S	IM	R	S	S	S	IM	S	S	S	S	S	S	S	S	nd	S	R	S	S	S	S	S	IM	S	S	S
16 Wayne	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	IM	S

^a Infection types were classified into three categories described in Akamatsu et al. (2013): R, white letters and black background; IM, white letters and gray background; and S, black letters and white background; nd (black letters and light gray background), infection type was not determined due to preparation failure involving certain differential plants.

^b The numbers correspond to those in Table 1.

Table 3. Infection types of 31 *Phakopsora pachyrhizi* samples from Brazil on 16 differentials ^a

Location no. ^c		1				2					3								5		6				7	8	9	10	11	12	15	16		
Season		2012/ 2013	2013/ 2014	2014/ 2015	2010/ 2011	2010/ 2011	2011/ 2012	2012/ 2013	2013/ 2014	2014/ 2015	2010/ 2011	2010/ 2011	2011/ 2012	2011/ 2012	2011/ 2012	2011/ 2012	2012/ 2013	2013/ 2014	2014/ 2015	2010/ 2011	2010/ 2011	2013/ 2014	2014/ 2015	2012/ 2013	2014/ 2015	2010/ 2011	2011/ 2012	2011/ 2012	2011/ 2012	2011/ 2012	2012/ 2013	2013/ 2014		
Sample code		BSO1-6	BSO1-7	BSO1-8	BSO2-4(1)	BSO2-4(2)	BSO2-5	BSO2-6	BSO2-7	BSO2-8	BSO3-4(1)	BSO3-4(2)	BSO3-5(1)	BSO3-5(2)	BSO3-5(3)	BSO3-5(4)	BSO3-6	BSO3-7	BSO3-8	BCW5-4	BCW6-4	BCW6-7	BCW6-8	BCW7-6	BCW8-8	BCW9-4	BCW10-5	BCW11-5	BCW12-5	BCW15-5	BCW15-7	BCW16-7		
1	PI 200492	R	nd	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	IM	S	S	S	S	S	S	S	
2	PI 368039	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	nd	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
3	PI 230970	R	IM	S	S	S	S	S	IM	IM	R	R	S	R	R	R	S	S	IM	S	R	IM	IM	S	S	nd	IM	IM	IM	IM	S	S	S	
4	PI 417125	R	R	S	S	S	S	S	S	S	IM	IM	S	R	R	R	S	IM	R	R	IM	S	IM	nd	S	IM	IM	S	S	IM	S	IM	IM	
5	PI 462312	nd	S	R	S	S	R	S	R	S	IM	S	S	R	R	R	S	S	IM	S	R	R	R	R	S	R	R	S	S	S	R	R	R	
6	PI 459025	R	R	S	S	S	R	S	R	S	R	R	R	R	R	R	S	R	S	nd	R	R	S	R	nd	S	R	S	IM	R	S	IM	R	
7	Shiranui	R	R	R	R	IM	R	IM	R	IM	R	R	R	R	R	R	IM	IM	R	R	R	R	R	R	R	nd	R	R	R	R	R	R	R	
8	PI 416764	R	nd	S	R	S	S	S	S	S	S	S	S	R	S	R	S	IM	R	R	IM	IM	S	R	IM	R	R	S	S	R	R	IM	S	
9	PI 587855	R	R	R	IM	R	IM	S	R	R	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	IM	R
10	PI 587880A	R	R	R	S	R	IM	S	R	R	S	R	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	
11	PI 587886	S	S	S	S	IM	S	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	
12	PI 587905	R	R	S	R	R	S	R	S	S	S	R	R	R	R	R	IM	IM	S	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
13	PI 594767A	R	R	R	R	R	S	R	IM	R	R	R	IM	R	IM	R	R	IM	R	R	R	R	R	IM	R	R	R	R	R	R	R	R	R	R
14	BRS 154	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
15	TK5	S	S	S	S	S	S	S	S	S	S	S	S	IM	S	S	S	S	S	S	IM	nd	S	S	S	S	S	S	S	S	S	S	S	
16	Wayne	S	nd	S	S	S	S	S	nd	S	S	S	S	S	S	S	S	nd	S	nd	S	S	S	S	S	S	S	S	S	S	S	S	S	S

^a Infection types were classified into three categories described in Akamatsu et al. (2013): R, white letters and black background; IM, white letters and gray background; and S, black letters and white background; nd (black letters and light gray background), infection type was not determined due to preparation failure involving certain differential plants.

^b The numbers correspond to those in Table 1

4. Pathogenic variation in rust populations from the three South American countries

A dendrogram constructed by cluster analysis was drawn based on the pathogenicity of 145 samples (i.e., 44

Argentinean, 58 Brazilian, 43 Paraguayan samples) and seven Japanese rust races from 2010/2011 to 2014/2015 (this study) and from 2007/2008 to 2009/2010 (Akamatsu et al. 2013) (Fig. 1). None of the *P. pachyrhizi* samples

Table 4. Infection types of 24 *Phakopsora pachyrhizi* samples from Paraguay and seven isolates from Japan on 16 differentials ^a

Location no. ^b	3			6				7	12	13						15						16		17	18	19	Ibaraki, Japan ^c											
Season	2010/2011	2011/2012	2014/2015	2011/2012	2012/2013	2013/2014	2014/2015	2010/2011	2010/2011	2010/2011	2011/2012	2012/2013	2013/2014	2014/2015	2010/2011	2011/2012	2012/2013	2013/2014	2014/2015	2012/2013	2014/2015	2012/2013	2014/2015	2014/2015	2014/2015	2014/2015	2014/2015	2014/2015	2014/2015	2007	2007	2008	2008	2007	2007	2009		
Sample code	PNC3-4	PNC3-5	PNC3-8	PMA6-5	PMA6-6	PMA6-7	PMA6-8	PMA7-4	PSI12-4	PSI13-4	PSI13-5	PSI13-6	PSI13-7	PSI13-8	PSI15-4	PSI15-5	PSI15-6	PSI15-7	PSI15-8	PNSI16-6	PNSI16-8	PNC17-8	PMA18-8	PMG19-8	T1-4	Race 1	N1-1	Race 2	N2-3	Race 3	T3-1	Race 4	E1-4	Race 5	K1-2	Race 6	K3-1	Race 7
1 PI 200492	S	S	S	S	S	R	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	
2 PI 368039	S	S	S	S	S	R	S	S	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	
3 PI 230970	IM	IM	S	R	R	R	IM	R	IM	IM	IM	S	R	S	R	S	S	S	S	S	S	IM	S	S	S	S	S	IM	R	R	R	R	R	R	R	R	R	
4 PI 417125	S	nd	S	S	S	IM	S	IM	R	R	R	IM	S	R	S	IM	IM	IM	S	S	IM	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	R	
5 PI 462312	IM	nd	S	S	R	R	R	nd	S	IM	R	R	R	R	S	S	S	R	S	IM	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
6 PI 459025	IM	R	R	nd	S	IM	R	IM	IM	R	R	R	R	R	IM	R	R	R	R	IM	S	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	
7 Shiranui	R	R	R	R	R	IM	R	R	R	R	R	R	R	R	R	R	R	R	IM	R	R	R	R	R	R	R	R	R	IM	R	R	R	R	R	R	R	R	R
8 PI 416764	S	R	R	R	R	IM	R	R	R	IM	S	S	R	R	R	S	R	IM	R	IM	R	R	IM	R	R	R	R	IM	R	R	R	R	R	R	R	R	R	
9 PI 587855	R	S	R	nd	R	R	R	R	R	nd	R	R	R	R	IM	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	nd	R	R	R	R	R	
10 PI 587880A	R	nd	R	R	R	R	R	R	nd	R	R	R	R	R	IM	S	S	R	S	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
11 PI 587886	S	S	S	S	R	R	S	S	nd	R	S	S	R	R	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
12 PI 587905	R	R	S	S	R	R	R	R	R	nd	R	R	R	R	R	R	R	S	R	S	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
13 PI 594767A	R	R	S	R	S	R	R	R	IM	R	R	R	S	R	R	R	S	R	R	R	IM	R	R	R	R	R	R	R	nd	R	R	R	R	R	R	R	R	R
14 BRS 154	S	S	S	S	S	S	S	S	S	S	S	S	IM	S	S	S	S	S	S	S	S	S	S	IM	S	S	S	S	S	S	S	S	S	S	S	S	S	S
15 TK5	S	nd	S	S	S	R	S	R	IM	R	nd	S	R	S	R	S	S	IM	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R
16 Wayne	S	S	IM	S	S	R	S	S	IM	IM	S	S	S	S	S	S	S	IM	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S

^a Infection types were classified into three categories described in Akamatsu et al. (2013): R, white letters and black background; IM, white letters and gray background; and S, black letters and white background; nd (black letters and light gray background), infection type was not determined due to preparation failure involving certain differential plants.

^b The numbers correspond to those in Table 1

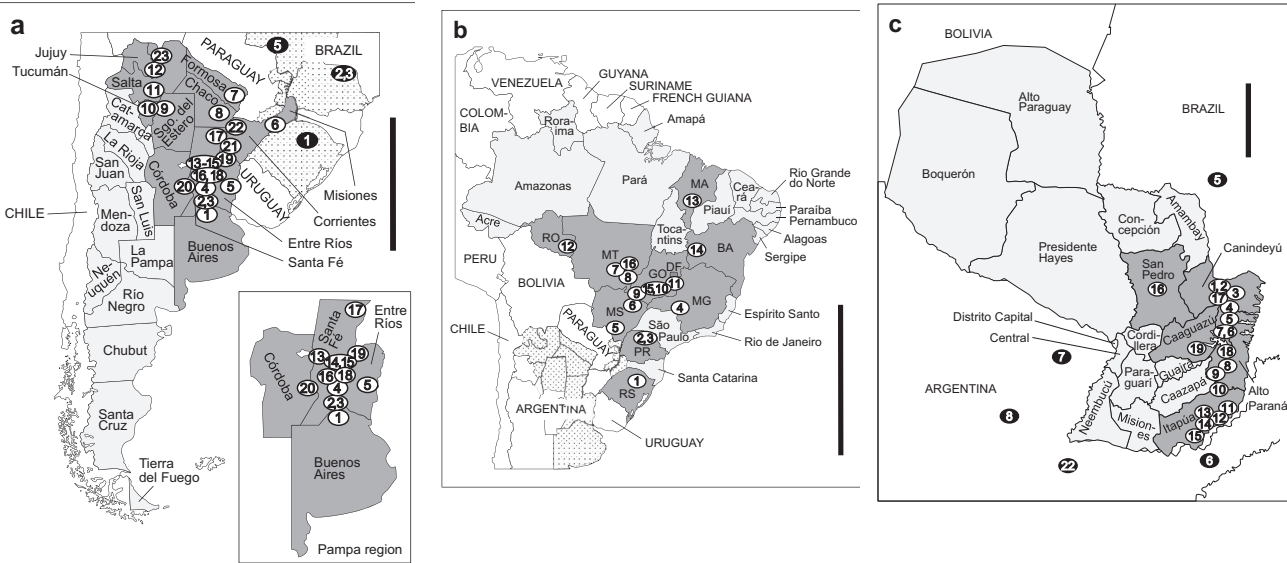


Fig. 1. Sampling locations of soybean rust in Argentina (a), Brazil (b), and Paraguay (c). Provinces of Argentina, states of Brazil, and departments of Paraguay are shown on each map. Provinces, states, and departments from which pathogen samples were obtained are indicated in dark gray; others are indicated in light gray. Indicated sampling location numbers correspond to Table 2. The black bars in Figs. 1a, 1b, and 1c, show 1,000 km, 2,000 km, and 200 km, respectively. Blank maps of the three countries were downloaded from <http://www.freemap.jp/>.

from the three countries during the 2010/2011 to the 2014/2015 seasons were identical in their pathogenicity profiles in the 16 differentials. Some South American rust samples from the 2010/2011 to the 2014/2015 seasons displayed the same pathogenicity profiles in the 16 differentials as those from the three previous seasons spanning 2007/2008 to 2009/2010: BCW16-7 and BSO1-3 both from Brazil (AU p-value = 100%), BSO3-5(2) from Brazil, and BSO4-2 and PMA5-3 from Brazil and Paraguay (AU p-value = 100%), respectively.

The 145 *P. pachyrhizi* populations from the three countries were scattered throughout the dendrogram, and only a few of the South American populations with the same country of origin formed subclusters. PMA6-7 and PSI13-7 demonstrated low pathogenicity in the differentials carrying *Rpp* genes, and together with PSI14-3 from the 2009/2010 season, were found in a subcluster containing the three Japanese rust races E1-4 (Race 5), K1-2 (Race 6), and K3-1 (Race 7) (AU p-value = 68%). In contrast, ANE6-6, BSO3-7, and PSI15-6 were the most virulent populations in Argentina, Brazil, and Paraguay, respectively, and fell into a subcluster (AU p-value = 92%). Furthermore, the neighboring subcluster contained the six populations expressing the highest virulence towards the differentials (AU p-value = 95%). Cluster analysis revealed no apparent geographical or seasonal association with pathogenicity.

5. Reactions of the *Rpp*-carrying differentials to *P. pachyrhizi* from South America

Fig. 2 indicates the frequencies of infection types to the soybean rust populations of the three countries of the 13 *Rpp*-carrying differentials. The differentials carrying *Rpp1* had the most cases of susceptible reactions and those carrying *Rpp1-b* the most cases of resistant reactions. The differentials carrying *Rpp2*, *Rpp3*, *Rpp3-?*, and *Rpp4* produced slightly resistant, moderately resistant, slightly resistant, and more resistant reactions, respectively. Shiranui (*Rpp5*) was resistant comparable to differentials carrying *Rpp1-b*.

The frequencies of resistant reactions in the differentials carrying *Rpp1*, *Rpp1-b*, and *Rpp2* were relatively consistent over the eight seasons (Fig. 3). In contrast, PI 462312 (differential 5, *Rpp3*) displayed more widely varied frequencies of resistant reactions among the seasons; none of the rust populations from the 2007/2008 season resulted in a resistant reaction, while 89% of the populations from the 2013/2014 season did. The other *Rpp3*-carrying differential (PI 416764) produced a resistant reaction in response to 5%-53% of the rust populations. The *Rpp4*-carrying differential (PI 459025) displayed higher proportions of resistant reactions in the

four seasons from 2011/2012 to 2014/2015 (50%-89%) compared with those from 2007/2008 to 2010/2011 (25%-40%). The frequency of resistant reactions in the *Rpp5*-carrying differential ranged from 50%-94%.

Although this study primarily focused on the

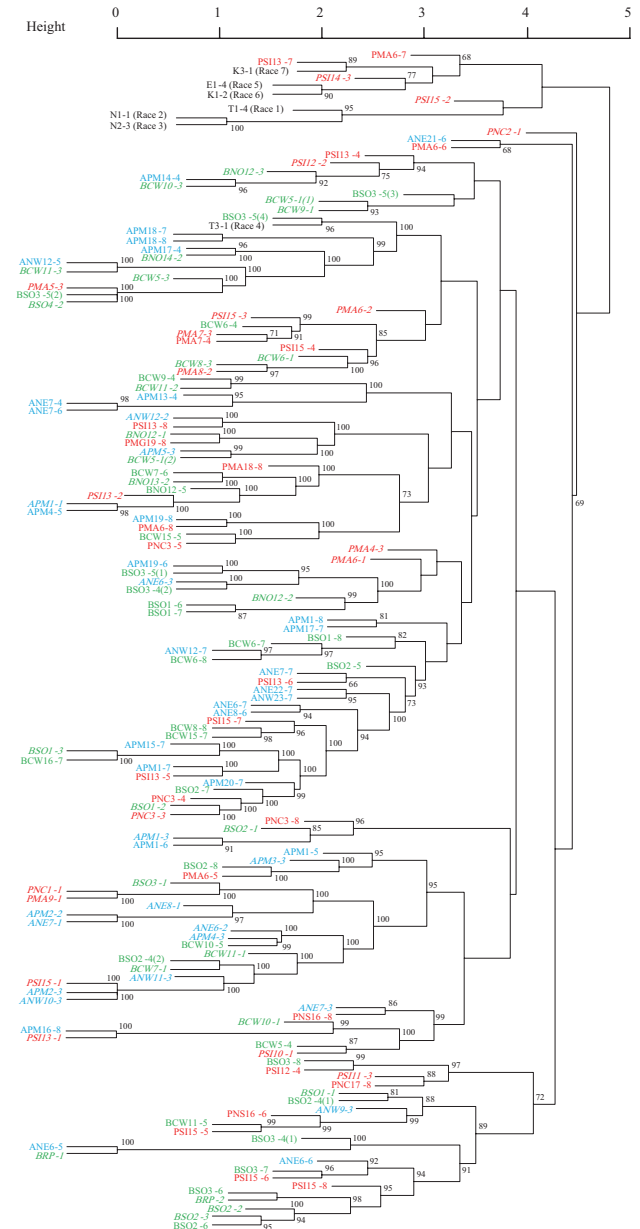


Fig. 2. Correlations of the 44 Argentinean, 58 Brazilian, and 43 Paraguayan soybean rust populations based on pathogenicity to the differentials.

The numbers at the nodes indicate AU p-values (> 65%) generated by 10,000 multiscale bootstrap resamplings. Rust populations from Argentina, Brazil, and Paraguay are indicated by blue, green, and red letters, respectively. Sampling seasons are denoted by different font styles: the 2007/2008-2009/2010 seasons in italics; the 2010/2011-2014/2015 seasons in regular font.

reactions for 16 differentials, of which 13 contained *Rpp1-5* and their associated alleles, the preliminary results demonstrate that PI 567102B carrying *Rpp6* (Li et al. 2012) and the No6-12-1 line pyramided with *Rpp2*, *Rpp4*, and *Rpp5* (Lemos et al. 2011) produced a resistant reaction to some recent rust populations from the three South American countries (data not shown).

Discussion

We have uncovered the pathogenic diversity in Argentinean, Brazilian, and Paraguayan rust populations collected during three cropping seasons from 2007/2008 to 2009/2010 (Akamatsu et al. 2013). The plant breeding strategies for developing disease-resistant cultivars largely depend on the pathogenicity of plant pathogens in the target region. Therefore, continuous monitoring of the pathogen populations while using the same method of evaluating pathogenicity as used before is important for elucidating the pathogenic variation of certain rust populations in South America. This study revealed that temporal and geographical pathogenic variations existed among *P. pachyrhizi* populations collected from the three countries in the 2010/2011 to 2014/2015 cropping seasons, and that geographical and temporal associations with pathogenicity of the rust populations are not apparent in South America. Furthermore, we also found that the proportions of resistant reactions in *Rpp1-b*-carrying and *Rpp5*-carrying differentials to the South American rust populations were higher than the eight other differentials. Moreover, this is the first report to monitor *P. pachyrhizi* pathogenicity over an extensive period, and across the

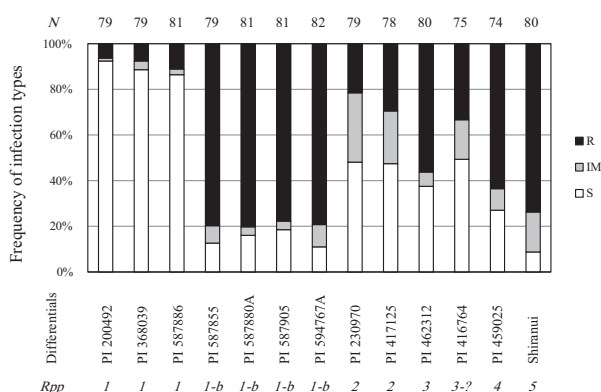


Fig. 3. Frequency of infection types produced in 13 differentials by inoculation with South American *P. pachyrhizi* populations during the 2010/11-2014/2015 seasons. The number of samples tested (*N*) is shown at the top of the plot. The *Rpp* genes that each differential has are shown at the bottom.

three countries in South America.

Pathogenic race studies have been conducted in South America ever since 2001 when soybean rust caused by *P. pachyrhizi* was first recorded in Paraguay and Brazil. Yorinori (2008) reported that *Rpp1-Rpp4* differentials that had been resistant in 2002 became susceptible in 2003. In the 2007/2008 season and later, *P. pachyrhizi* populations induced a susceptible reaction in the differentials carrying *Rpp1-Rpp4* in Argentina, Brazil, and Paraguay (Akamatsu et al. 2013). Similarly, a *P. pachyrhizi* isolate from Taiwan collected in 1972 produced a resistant reaction in an *Rpp2*-carrying PI, and an isolate collected in 1980 caused a susceptible reaction in the same PI (Bonde et al. 2006, Pham et al. 2009). Bonde et al. (2006) reported that *P. pachyrhizi* isolates collected in South America, Africa, and Thailand in 2001 were more virulent and/or aggressive than older isolates

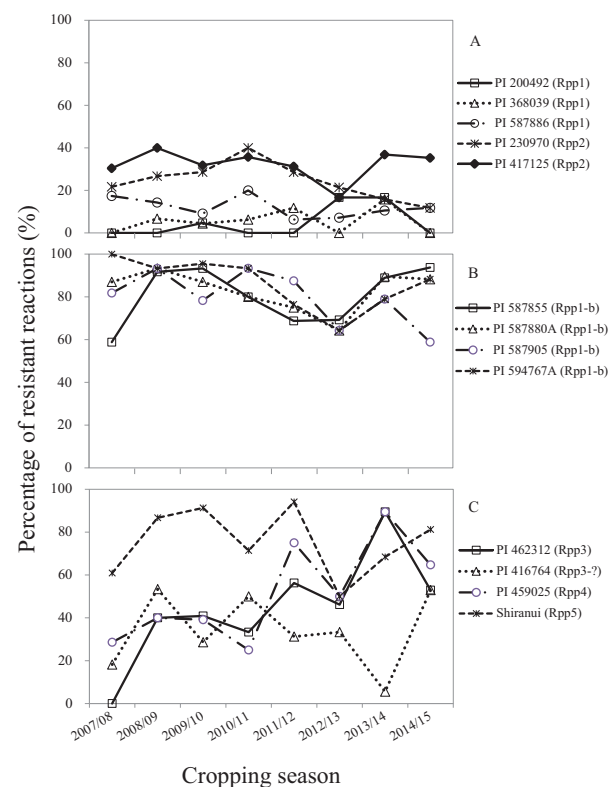


Fig. 4. Frequency of the resistant responses produced in 13 differentials by inoculation with South American *P. pachyrhizi* populations during the 2007/2008-2014/2015 seasons.

A: *Rpp1*-carrying differentials of PI 200492, PI 368039, and PI 587886; and *Rpp2*-carrying differentials of PI 230970 and PI 417125. B: *Rpp1-b*-carrying differentials of PI 587855, PI 587880A, PI 587905, and PI 594767A. C: PI 462312 (*Rpp3*), PI 416764 (*Rpp3-?*), PI 459025 (*Rpp4*), and Shiranui (*Rpp5*).

collected from 1972 to 1994.

The temporal change of pathogenic profiles was also reported in the US. The US isolates expressing the same pathogenicity profile as US isolates collected in 2004 when soybean rust was first found in the US (Schneider et al. 2005), have not been detected in subsequent US samples (Paul et al. 2015, Paul & Hartman 2009, Pham et al. 2009). Therefore, it has been suggested that *P. pachyrhizi* isolates existing in 2004 disappeared or were suppressed due to the subsequent winter, likely resulting in unfavorable conditions for pathogen survival (Paul et al. 2015, Paul & Hartman 2009). In this study, a highly virulent Argentinean population (i.e., ANE6-6) was discovered, and it could pose a serious threat since none of the *Rpp*-carrying differentials demonstrated a resistant reaction to the population. Fortunately, such rust populations were never found after the 2012/2013 season, and thus were unlikely to survive the subsequent winter and further expand.

During the 2007/2008 to 2009/2010 seasons, South American rust populations never resulted in a susceptible reaction in PI 594767A carrying *Rpp1-b* (Akamatsu et al. 2013). However, rust populations overcoming the resistance to the same PI were later identified in Argentina, Brazil, and Paraguay. This study has thus resulted in the accumulation of valuable data regarding pathogenic variation, likely reflecting the diversity of the pathogen races or pathotypes in these countries. Furthermore, this study represents a contribution to the detection of sudden changes in pathogenicity and whether novel pathogen populations are likely to expand or contract.

The data obtained will enable a comparison of pathogenicity between the US and South American populations, but cannot be compared directly as the data were obtained using different evaluation methods. The data revealed a large difference in the rust reactions of *Rpp1*-carrying PI 200492 and PI 587880A; PI 587880A possesses a different *Rpp1* allele compared with PI 200492, *Rpp1-b* (Ray et al. 2009, Yamanaka et al. 2015a). The reactions of PI 200492 and PI 587880A to most South American rust populations were evaluated as being susceptible and resistant, respectively, whereas those to the US isolates were evaluated as being resistant and susceptible, respectively (Paul et al. 2015, Twizeyimana & Hartman 2012). In contrast, the *P. pachyrhizi* isolates collected in Alabama in 2004 induced a susceptible reaction in *Rpp1*-carrying PI 200492. Pham et al. (2009) confirmed that the rust isolates resulting in a susceptible reaction in PI 200492 already existed in Brazil by 2001. These findings suggest that initial entry of the pathogen was achieved by its long distance movement

from Central or South America (Isard et al. 2005, Pan et al. 2006). In addition, the pathogen genotype that could defeat *Rpp1* in PI 200492 was restricted by the subsequent winter season, lost, and then never re-established in the US, despite urediniospores of such populations that could be reintroduced from Central or South America. A comparative study following the evaluation of *P. pachyrhizi* populations from both the US and Central America (e.g., Mexico) would establish more comprehensive data to determine the variability and correlation of the pathogen populations between North and South America.

This work elucidates the pathogenicity of *P. pachyrhizi* populations from Argentina, Brazil, and Paraguay from the 2010/2011-2014/2015 seasons, and provides a dataset useful for comparing pathogenicity data obtained in the past and for the future monitoring of pathogen populations. In an on-going study, the pathogenicity of the *P. pachyrhizi* populations from the three countries has been evaluated using the differentials with *Rpp1-5* and their additional alleles, PI 567102B carrying *Rpp6* (Li et al. 2012) and the No6-12-1 line pyramided with *Rpp2*, *Rpp4*, and *Rpp5* (Lemos et al. 2011). In the future, a more informative dataset containing infection types of the two new differentials would be useful in breeding programs to develop rust resistant cultivars for *P. pachyrhizi* populations outside of Argentina, Brazil, and Paraguay (e.g., Central America). To our knowledge, this paper, coupled with Akamatsu et al. (2013), describes the pathogenicity of the largest *P. pachyrhizi* collection, consisting of populations from eight cropping seasons from 2007 to 2015 in three South American countries.

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